Fig. 1

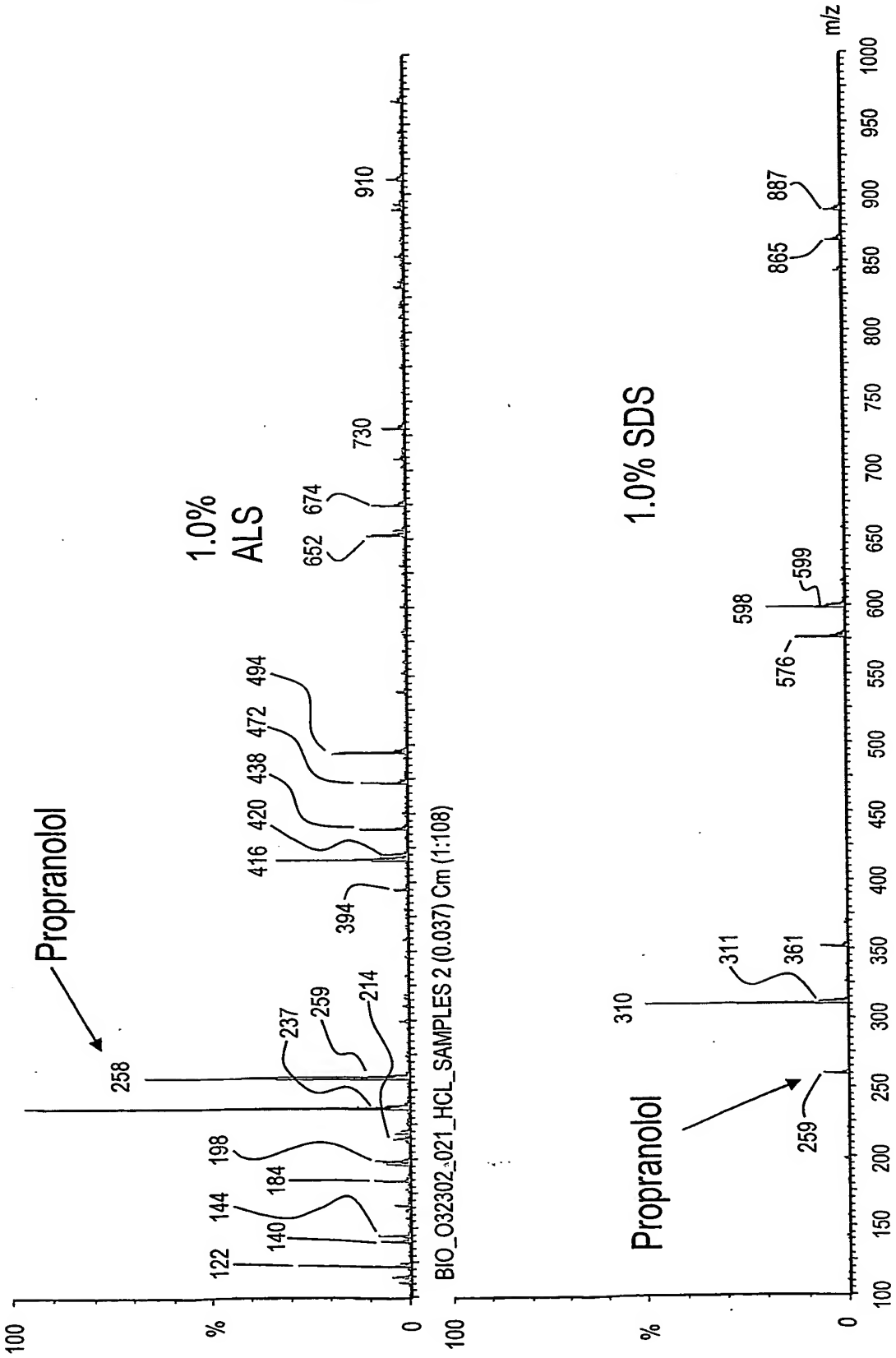
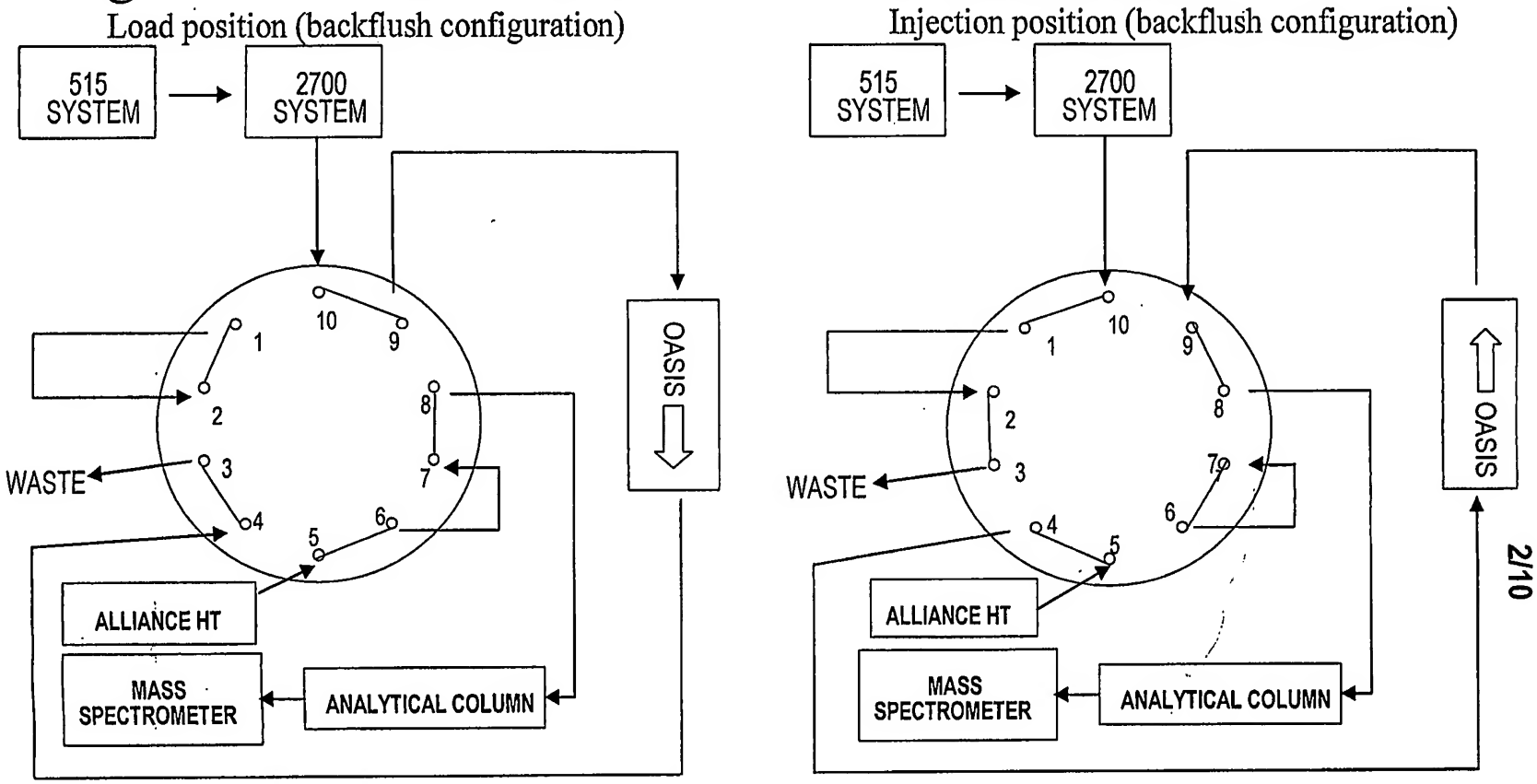

BIO_032302_013_HCL_SAMPLES 85 (1.560) Cm (1:108)
Propranolol
1.0% ALS
BIO_032302_021_HCL_SAMPLES 2 (0.037) Cm (1:108)
1.0% SDS
Propranolol
%
100
0
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
122
140
144
184
198
214
237
258
259
310
311
361
394
416
420
438
472
494
576
598
599
652
674
730
865
887
910

Fig. 2 **Oasis® HLB/XTerra® Columns Configuration**



LC_1: Alliance 2690 - 0.4 mL/min
LC_2: Waters 515 - 4.0 mL/min
Loading mobile phase: 100 % water
Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN
Eluting mobile phase additive: 0.5 % Formic acid
Extraction column temperature: room temperature
Switching valve: Rheodyne LabPro 10 ports, 2 position

MS: Quattro Ultima Triple Quadrupole
Source: Electrospray positive
Source temperature: 150 °C
Desolvation gas: 600 L/hr
Gas cell: 2.0e-3 mbar
Cone Voltage: 20 volts
Collision energy: 20

PCT/US03/16819
10/516419

Fig. 3

HPLC Gradient and Wash Conditions

Time	HPLC gradient Flow 0.4 mL/min		Valve position	Function
	A	B		
0.0	5	95		
0.5	5	95	position 1	Loading with 100 % H_2O
1.5	95	5	position 2	Elution with 1 min gradient
4.40	95	5		
4.50	5	95	position 1	Return to loading position
6.0	5	95		

A - Acetonitrile + 0.5 % Formic Acid
B - Water + 0.5 % Formic Acid

10/516419

Fig. 4

50/50 MeOH/ACN Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL

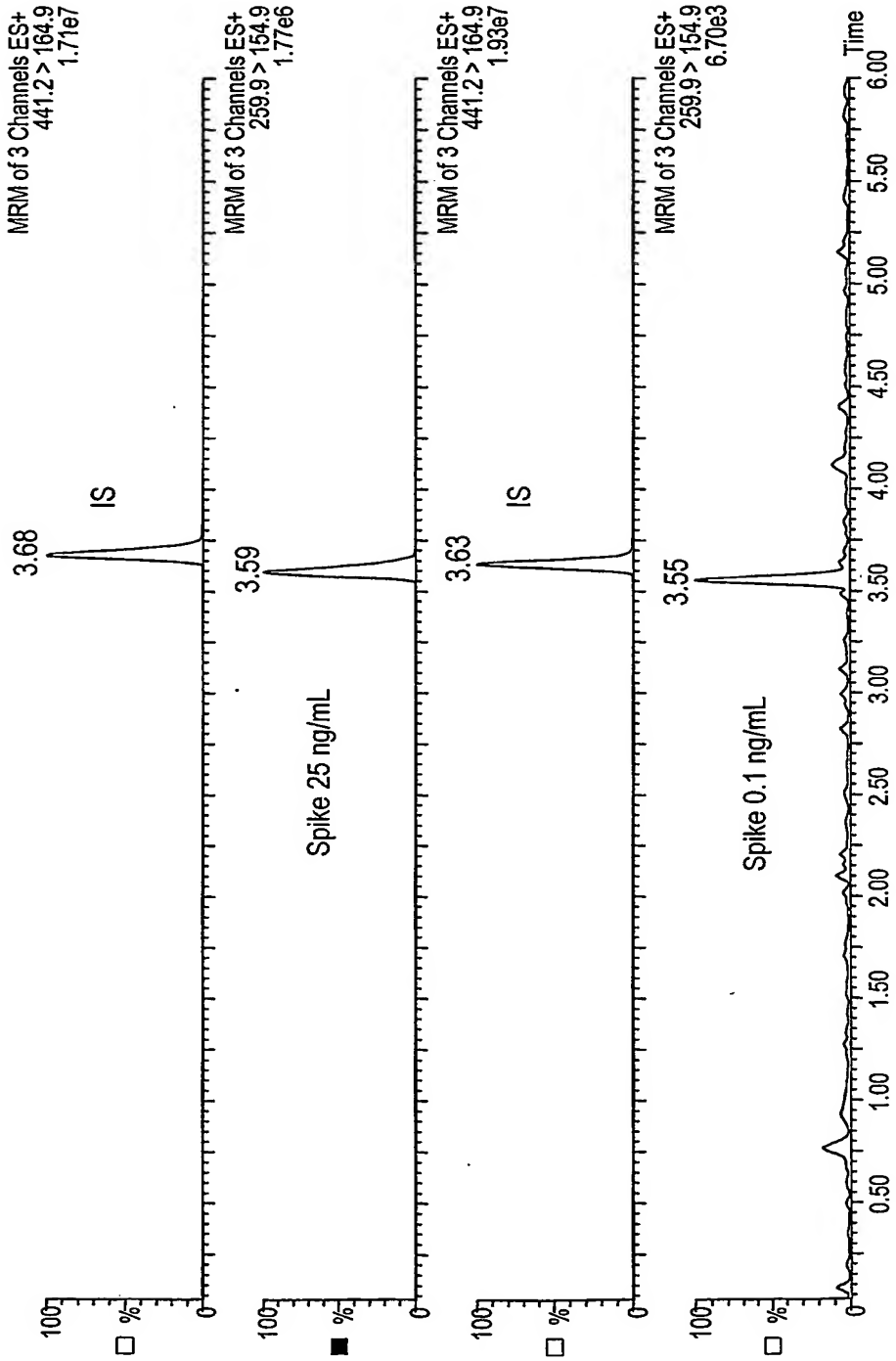


Fig. 5 **Ion Suppression of Surfactants**

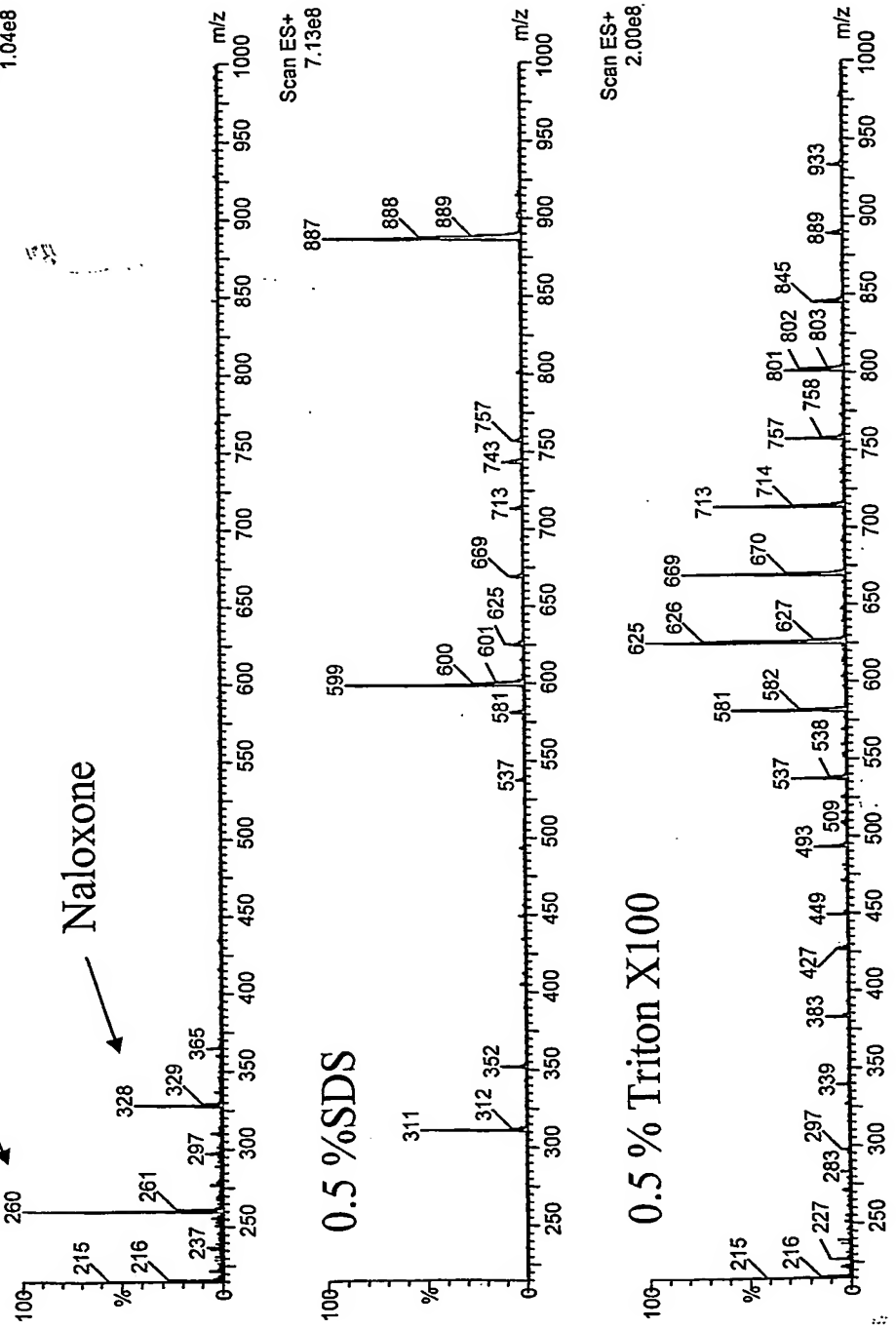
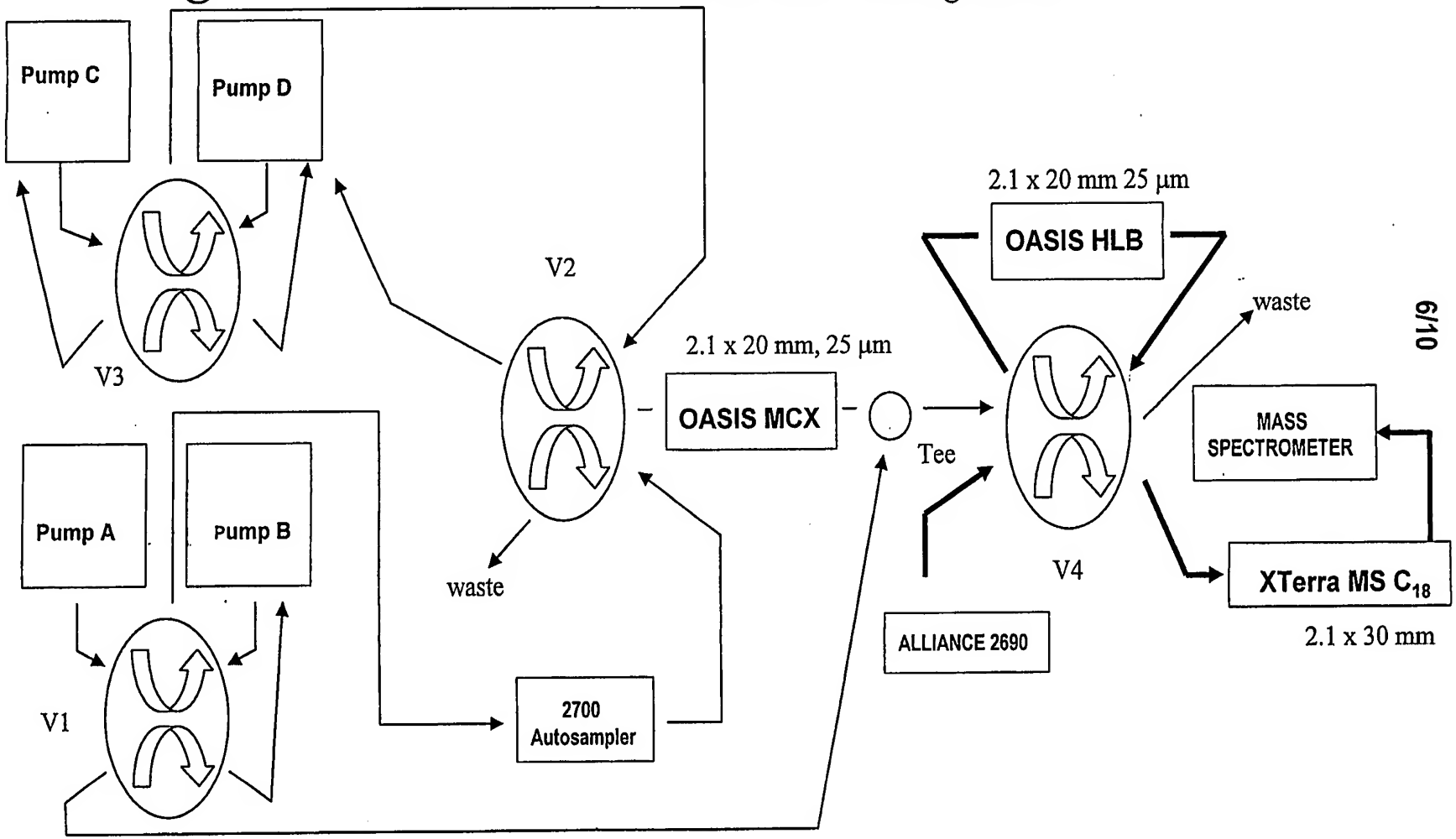


Fig. 6 **Oasis® HLB / MCX / XTerra® Configuration**



Pump A: 100 % water + 2 % NH_4OH
Pump C: 100 % Water + 4 % Formic Acid

Pump B: 100 % Methanol
Pump D: 100 % Methanol + 2 % NH_4OH

PCT/US03/16819

10/516419

Fig. 7

HPLC Gradient and Wash Conditions

Time (min)	HPLC gradient Flow 0.4 mL/min		Valve Position V1, V2, V3, V4	Function
	A	B		
0.0	5	95	2 – 2 – 2 – 2	Load 100 % H_2O pH 11
1.0	5	95	1 – 1 – 2 – 2	Wash 100 % H_2O pH 2
2.0	5	95	1 – 2 – 2 – 2	Wash (see chromatograms)
3.0	5	95	1 – 1 – 1 – 2	Elution of MCX onto HLB (pH 11)
4.0	5	95	1 – 1 – 1 – 1	Elution of HLB onto Xterra (pH 3)
5.0	95	5		
7.0	95	5		
7.5	5	95		
8.0	5	95	2 – 2 – 2 – 2	Reset to starting position
9.0	5	95		

A - Acetonitrile + 0.5 % Formic Acid

B - Water + 0.5 % Formic Acid

PCT/US03/16819
10/516419

Fig. 8

1 % Triton X100 Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL

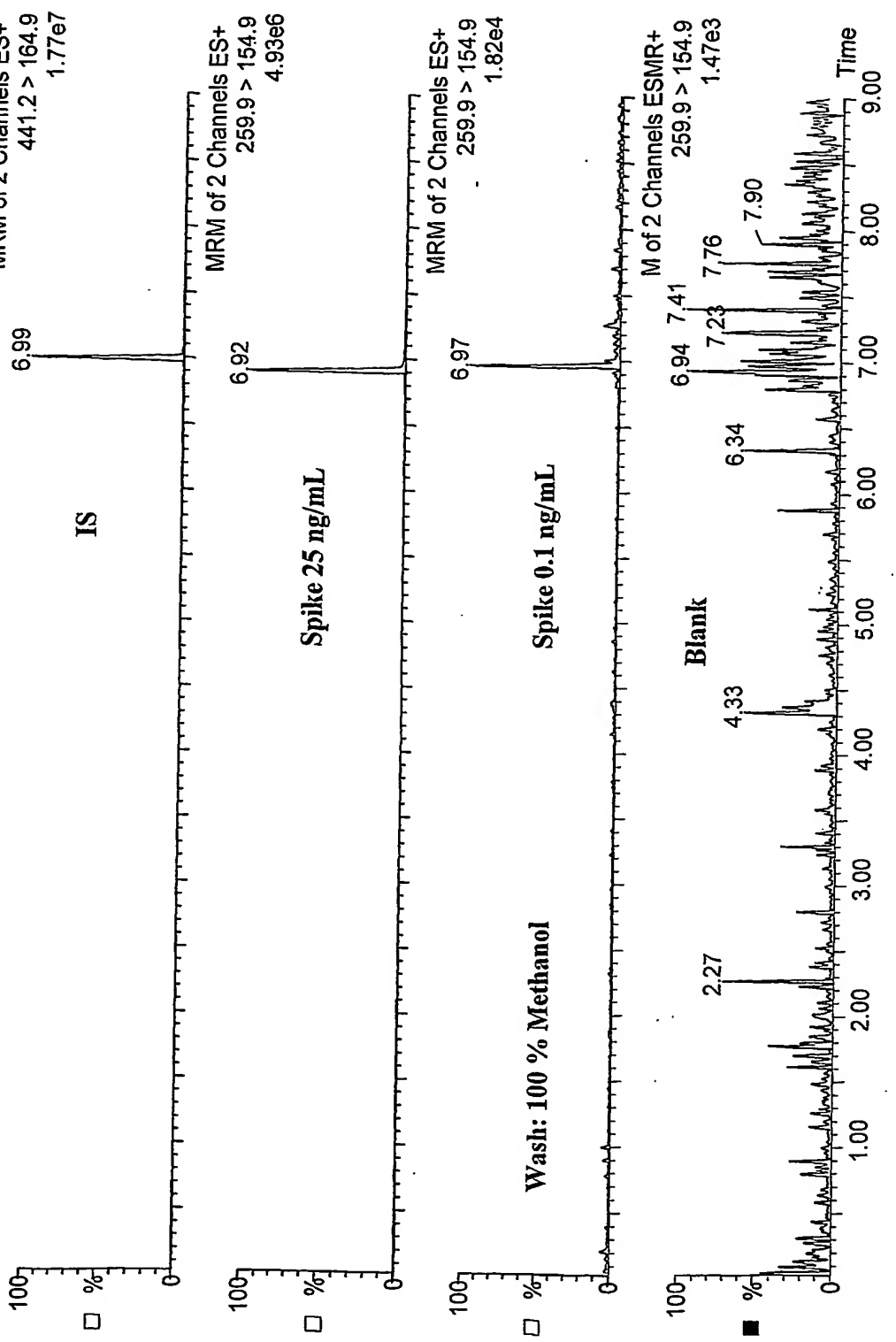


Fig. 9

1 % SDS Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL

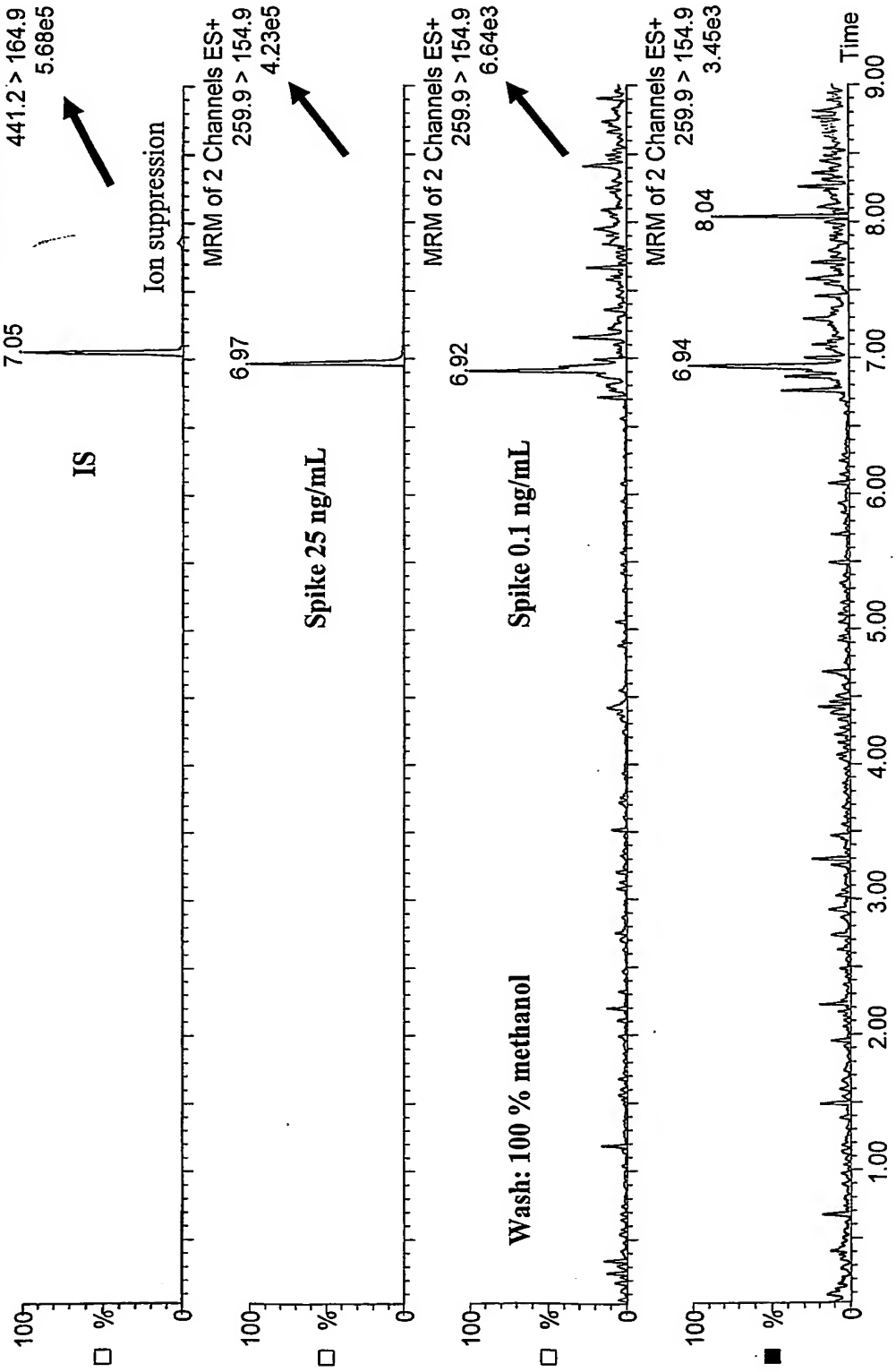
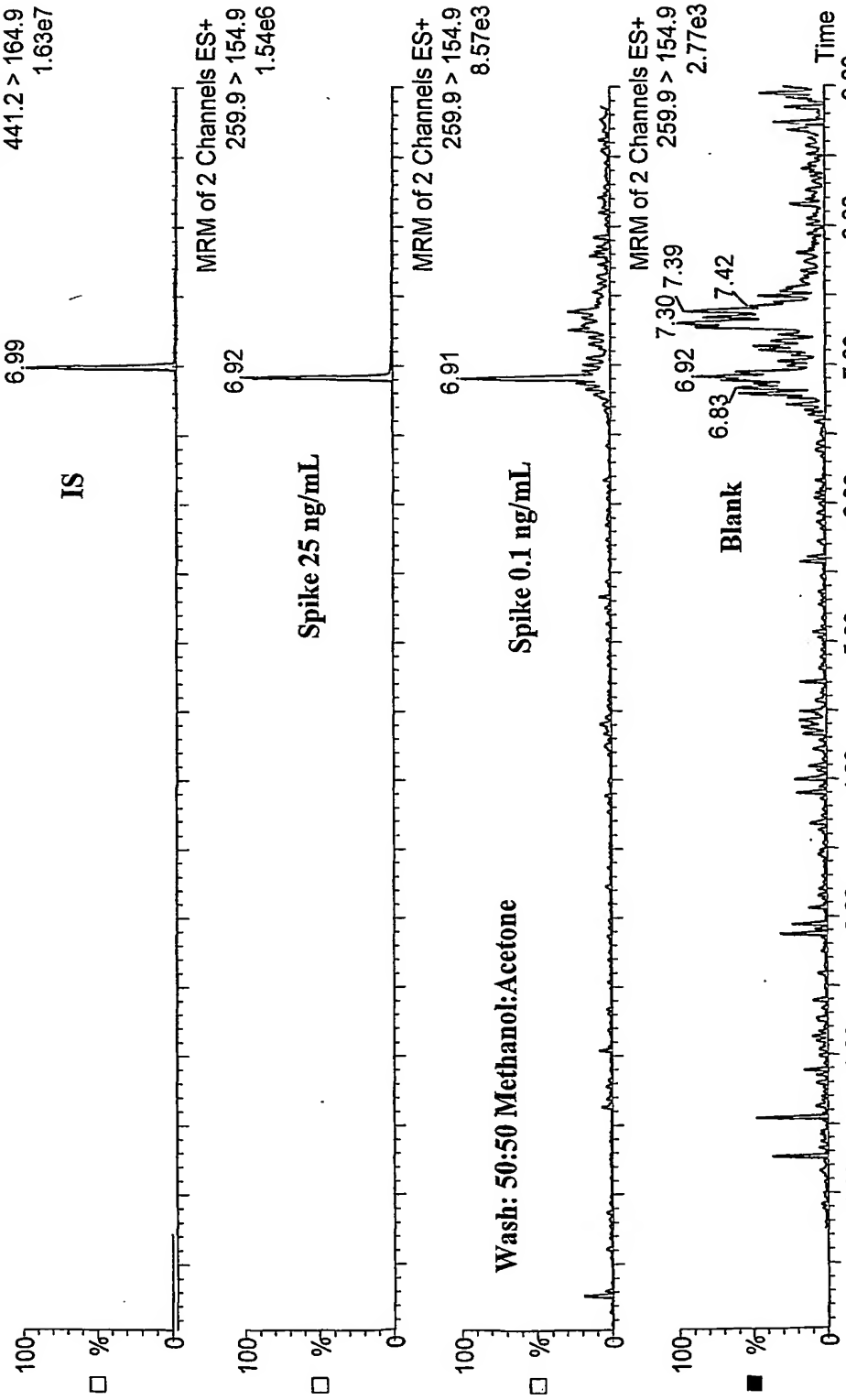


Fig. 10


1 % SDS Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL
IS
MRM of 2 Channels ES+
441.2 > 164.9
1.63e7
6.99
Spike 25 ng/mL
MRM of 2 Channels ES+
259.9 > 154.9
1.54e6
6.92
Spike 0.1 ng/mL
MRM of 2 Channels ES+
259.9 > 154.9
8.57e3
6.91
Blank
MRM of 2 Channels ES+
259.9 > 154.9
2.77e3
6.83
6.92
7.30
7.39
7.42
100
%
0
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Time